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RESEARCH ARTICLE OPEN ACCESS

Prevalence of Transferable OXA-1 β-Lactamase Associated with Carbapenem-Resistant *Klebsiella pneumoniae* Isolates in Iraq

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Abstract

This study was designed to explore the incidence of *bla*$_{OXA-1}$ amongst *Klebsiella pneumoniae* isolates with resistant to carbapenem. Between December 2014 and April 2015, one hundred samples were taken from two hospitals: Babylon Teaching Hospital for Maternity and Pediatric / Babylon Province (clinical, umbilical infections, n= 40; environmental, n=20) and Karbala Hospital for Pediatric / Karbala Province (40 stool samples). All patients were hospitalized or attended these hospitals, all under 1 year of age. Seventeenth (17%) isolates were identified as *Klebsiella pneumoniae*. The antibiotic resistance profile of isolates was tested using disk diffusion method. High-level of resistance was recorded with ampicillin (94.1%) and piperacillin (88.2%) antibiotics. Resistance to carbapenem was reported in two *K.pneumoniae* isolates, these were investigated for the existence of OXA-1β-lactamase using Polymerase Chain Reaction (PCR) technique. Two (100%) isolates gave positive result. Transference of this gene was studied by conjugation experiment. The *bla*$_{OXA-1}$ gene conjugated successfully in 1 (50%) isolate only.

Keywords: *Klebsiella pneumoniae*, Carbapenem resistance, OXA-1 β-lactamase, PCR, Conjugation

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INTRODUCTION

Antimicrobial resistance is a major public health problem worldwide. Infections caused by multi-drug resistance organisms due to long hospital stay, antibiotics treatment and poor hygiene are in continuous increase and linked with high rates of mortality and morbidity [1,2]. The possible resistance mechanism in *Klebsiella* spp is the production of extended spectrum beta-lactamases (ESBLs). These enzymes are capable of hydrolyzing penicillin, cephalosporin (3rd and 4th generation), monobactams, but have no effect on cephamycins or carbapenem[3,4] .

The predominant mechanisms for resistance to inhibitor penicillin combinations are : class C chromosomal β-lactamase production, overproduction of TEM-1 and TEM-2 type β-lactamases and OXA-1 β-lactamase production[5,6,7].

OXA-1 β- lactamase has the ability to hydrolyzes amino, ureidopenicillins (piperacillin),cloxacillin, oxacillin and methicillin in significant mean while it hydrolyzes cephalosporins (narrow –spectrum) weakly. Moreover, it hydrolyzes broad-spectrum cephalosporins, mediated diminished susceptibility to antibiotics like cefepime and cefpirome[8,9]. OXA-1 β- lactamase distributed widely among *Enterobacteriaceae* family and a major reason for resistance to amoxicillin /clavulanic acid combination mainly in *Escherichia coli* and *Salmonella enterica*[10,11,12].

The present work was attempted to evaluate the frequency of *Klebsiella pneumoniae* among clinical and environmental specimens, characterize resistant isolates, detect bla_{OXA-1} gene using Polymerase Chain Reaction (PCR) technique in isolates showed resistance to carbapenem and test its transmissibility by conjugation experiment.

MATERIALS AND METHODS

Sample collection

In a five months period (December,2014 to April, 2015), 100 different specimens were recovered from two hospitals namely :Babylon Teaching Hospital for Maternity and Pediatric / Babylon Province (clinical: umbilical infections, n=40; environmental: n=20) and Karbala Hospital for Pediatric / Karbala Province (40 stool samples). Collected samples were cultured on different prepared media. Suspected *K.pneumoniae* isolates were identified based on their colonial, morphological characteristics and microbiological procedures as mentioned previously[13,14,15].

Antimicrobial susceptibility testing

To determine the resistance profiles of *K.pneumoniae* isolates, the antimicrobial susceptibility to thirteen antimicrobial agents were analyzed by Kirby –Bauer disk diffusion method on plates with Mueller- Hinton agar medium (Oxiod, England)[16]. The selected agents included: ampicillin (AMP), piperacillin (PRL), amoxicillin- clavulanic acid (AMC), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), cefepime (FEP), cefoxitin (FOX), gentamicin (CN), imipenem (IMP), meropenem (MEM), levofloxacin (LE[5]) and norfloxacin (NOR). The results of susceptibility were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[17]. The *Escherichia coli* ATCC 25922 (University of Kufa, College of Medicine,) was used as the control strain in antimicrobial susceptibility testing.

Molecular detection of bla_{OXA-1} gene

Deoxyribonucleic acid (DNA) of carbapenem resistant *K.pneumoniae* was extracted based on the method mentioned with some modifications[18]. Conventional Polymerase Chain Reaction technique was applied to amplification bla_{OXA-1} gene using specific primers (Bioneer, Korea) OXA-1/F (F: ATA TCT CTA CTG TTG CAT CTC C) and OXA-1/R (R: AAA CCC TTC AAA CCATCC) (619 bp). All amplifications were implemented in a total volume of 25 µl consisted of 12.5 µl Go Taq Green Master Mix 2X (Promega, USA), 5 µl of extracted DNA, 2.5 µl forward and reverse primer (10 pmol/ µl) each and 2.5 µl nuclease-free water. The DNA template was denatured at 94°C for 5 min, followed by 30 cycles of denaturation (94°C for 50 sec), annealing (55°C for 50 sec), extension (72°C for 1 min) and the final extension (72°C at 10 min)[19] . The PCR reaction product was separated by gel electrophoresis (1.5% agarose gel stained with ethidium bromide solution, 0.5 mg/ml) at 70 volts for 2-3 hrs, PCR product was examined using UV-Transilluminator, and photographed with Gel documentation system. The size of DNA band was estimated using DNA Ladder, 100 bp (Bioneer, Korea).

Conjugation experiment

To test the transmissibility of bla_{OXA-1} gene, two carbapenem-resistant *K.pneumoniae*

harboring OXA-1 gene (donors) and rifampicin resistant *Escherichia coli* MM294 (University of Kufa, College of Medicine) (recipient),were selected. Conjugation experiment was attempted by liquid mating assay[20,21]. All the transconjugants were screened for the existence of this gene using PCR assay with same primers applied in the procedure. The Minimum inhibitory concentrations (MICs) for ampicillin , cefotaxime, ceftazidime, imipenem and meropenem were detected using HiComb Minimum Inhibitory Concentration (HiComb MIC) (Himedia, India) and Minimum Inhibitory Concentration Evaluator (M.I.C.E) (Oxoid, England) tests in accordance with the guidelines of Clinical and Laboratory Standards Institute[17].

RESULTS AND DISCUSSION

During study period (From December,2014 to April, 2015), 17(17%) strains were belonged to *Klebsiella pneumoniae* ,12 (70.6%) were recovered from stool samples and 5(29.4%) from umbilical infections, (Table 1). Recently, *K.pneumoniae* from stool samples was documented among children attending different hospitals in Dar es Salaam, Tanzanid[22] . Another report identified 12(2%) prevalence rate for *Klebsiella* spp. isolated from newborns with omphalitis in Pakistan[23].

However, *K.pneumoniae* from environmental samples was not detected in this study. The reason may be related to low number of tested samples. One study in Hillah city identified the species in various clinical and environmental samples[24]. Also, Abbas[25] proved the detection of *K.pneumoniae* from burn unit environment of Al-Hillah teaching hospital.

In this study, all *K.pneumoniae* isolates presented higher resistance against penicillin antibiotics (ampicillin, piperacillin) with (94.1%) and (88.2%) resistance rates, respectively, (table

Table 1. Prevalence of 17 *K.pneumoniae* isolates obtained from various samples

Hospital's name	Source of samples	Types and samples No.	*K.pneumoniae* isolatesNo.(%)
Babylon Teaching Hospital for Maternity and Pediatric	Clinical	Umbilical infections (n=40)	5 (29.4%)
	Environmental	Floor (n=10)	0 (%0)
		Beds (n= 6)	0 (%0)
		Walls (n=4)	0 (0%)
Karbala Hospital for Pediatric	Clinical	Stool (n=40)	12(70.6%)
Total		100	17 (100)

Table 2. Resistance profiles of the 17 *Klebsiella pneumoniae* isolates

Antimicrobial class	Antimicrobial tested	Resistant isolates No.(%)
Penicillins	Ampicillin	16(94.1%)
	Piperacillin	15(88.2%)
β –lactams "β- lactamase inhibitor combinations	Amoxicillin-clavulanic acid	14 (82.3%)
Cephems	Cefotaxime	13 (76.5%)
	Ceftazidime	12 (70.6%)
	Cefriaxone	1 2(70.6%)
	Cefepime	14(82.4%)
	Cefoxitin	13(76.5%)
Aminoglycosides	Gentamicin	10(58.8%)
Penems	Imipenem	2 (11.8%)
	Meropenem	2(11.8%)
Quinolones	Levofloxacin	4 (23.5%)
	Norfloxacin	4 (23.5%)

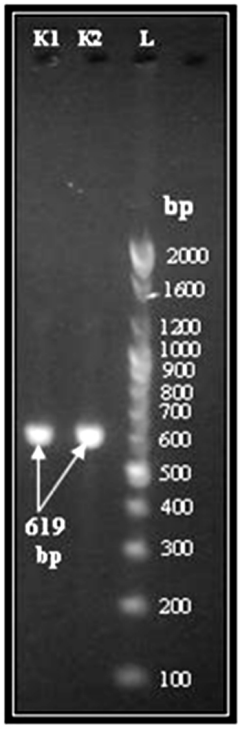

Fig. 1. Conventional PCR for amplification bla_{OXA-1} gene in *K.pneumoniae* isolates with resistant to carbapenem. Lane (L), 100- bp DNA Ladder. Lane (1,2) are OXA-1 positive isolates for bla_{OXA-1} gene (619 bp)

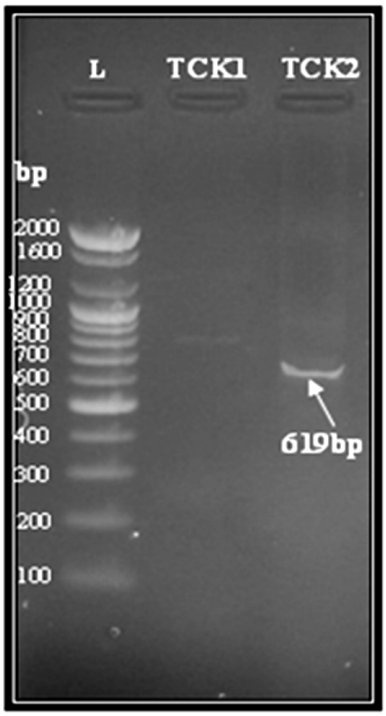

Fig. 2. Agarose gel electrophoresis for bla_{OXA-1} gene (619 bp) of transconjugant *E.coli* isolates TCK1 and TCK2. Lane (L), DNA molecular weight marker (100- bp Ladder). Lane (TCK2) showing positive result with bla_{OXA-1} gene. Lane (TCK1) showing negative result

-2). One work documented high level of resistance (98.6%) for ampicillin by *K.pneumoniae* among septicemic patients in India[9]. The higher frequency of resistance can be attributed to excessive consumption of these drugs in clinical settings.

Additionally, the lower frequency was observed with imipenem (11.8%) and meropenem (11.8%), (Table 2).One report carried out by Hashemi *et al*.[26] documented (20%) as a rate of resistance against imipenem and meropenem for *K.pneumoniae* isolated from two hospitals in Tehran, Iran. Other research characterized (28.57%) resistance rate for meropenem antibiotic by clinical isolates of *K.pneumoniae* in Southeastern Nigeria[27].

All carbapenem- resistant *K.pneumoniae* were positive for OXA-1 gene using PCR technique (Fig.1). According to Flores *et al*.[28] bla_{OXA-1} gene was

Table 3. Characteristics of carbapenem-resistant *K.pneumoniae* clinical isolates and their conjugates

Isolate	OXA-1 β-lactamase confirmed by PCR	MIC (µg /ml) AMP (>32)	CTX (>64)	CAZ (>32)	IMP (≥16)	MEM (≥16)
K. pneumoniae K1 (clinical isolate)	+	> 256	>240	>256	>32	>32
E.coli transconjugant(TCK1)	-	-	-	-	-	-
K. pneumoniae K2 (clinical isolate)	+	> 256	>240	>256	>32	>32
E.coli transconjugant(TCK2)	+	> 256	>240	>256	2	2

demonstrated in 42(60%) *K.pneumoniae* isolated from rectal swabs of patients settings intensive care unit, Brazil. The occurrence of *K.pneumoniae* harboring bla_{OXA-1} gene (34.4%) was previously reported in Malaysia[29].

Conjugation has been regarded as a very efficient method for horizontal transfer of resistance genes in bacteria with higher frequency in nature than under laboratory conditions[30,31]. In current research, conjugative transfer of bla_{OXA-1} gene was successful for only 1 (50%) isolate of *K.pneumoniae* (K2) which was selected as a donor for conjugation (Table-3,Fig.2).Successful transfer of OXA-1 gene by conjugation was previously reported in a spanish isolates of *K.pneumoniae*[32]. Also, Rakotonirino *et al*.[33] documented the transfer of this gene in 6 isolates of *K.pneumoniae* obtained from four hospitals and medical centers in Antananarivo, Madagascar. The widespread of OXA-1 gene may be related to localization of this gene on variable region of integrin (class I) that also contain other resistance determinants like *aac(6) Ib*, CTX-M ESBL type and carbapenemases[32].

Antibiotics susceptibilities of the transconjugant (TCK2) revealed higher resistance to ampicillin, cefotaxime and ceftazidime as its donor isolates with MIC values (>256, >240, >256) µg/ml, respectively,(table-3).Such higher resistance among transconjugant may be explained that OXA-1 gene was transferred and expressed .

The MIC of imipenem and meropenem for transconjugat (TCK2) was relatively lower (2 µg /ml) than the donor isolate (table -3).This finding suggest the presence of other resistant mechanism like chromosomal -mediated resistance genes in the donor that can not transferred by conjugation .

CONCLUSION

The current study documents the presence of *K.pneumoniae* carrying ESBL of OXA-1 gene .The ability of this gene for transference pose a significant challenge for therapeutic options currently in use. Therefore, effective prevention and control strategies must be applied to prevent occurrence and dissemination of these strains.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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